UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: 14-MAR-2013

SUBJECT: **Glyphosate.** Review and generation of Data Evaluation Record

PC Code: 417300
Decision No.: 468347
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0056606
MRID No.: 48865101, 48865102, 48865103, 48865104, 48865105

DP Barcode: D409997
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: 1071-83-6
40 CFR: 180.364

FROM: Monique M. Perron, S.D.
Toxicologist, Risk Assessment Branch 1
Health Effects Division (HED) (7509P)

THROUGH: Dana Vogel, Deputy Director
Health Effects Division (HED) (7509P)

TO: Carissa Cyran, Risk Review Manager
Pesticide Re-evaluation Division

I. CONCLUSIONS

RAB1 has reviewed the two generation reproduction toxicity study and it is an acceptable/guideline study.

II. ACTION REQUESTED

PRD requested a review of the glyphosate two generation reproduction toxicity study.

10920

DATA EVALUATION RECORD

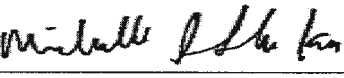
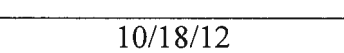
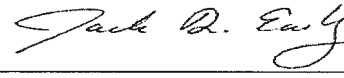
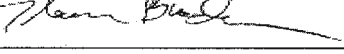
GLYPHOSATE

Study Type: OCSPP 870.3800; Reproduction and Fertility Effects Study

EPA Contract No. EP10H001452
Task Assignment No. 3-24-2012 (MRIDs 48865101 - 48865105)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Dynamac Corporation
1910 Sedwick Road,
Building 100, Suite B
Durham, NC 27713

Primary Reviewer: Michelle J. Sharpe-Kass, M.S.	Signature: Date:	 10/08/12
Secondary Reviewer: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Date:	 10/18/12
Program Manager: Jack D. Early, M.S.	Signature: Date:	 10/23/12
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature: Date:	 10/22/12

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

2

Primary Reviewer: Monique Perron, S.D. **Signature:** Monique Perron
Risk Assessment Branch 1, Health Effects Division (7509P) **Date:** 3/14/13
Secondary Reviewer: Anwar Y. Dunbar, Ph.D. **Signature:** Anwar Y. Dunbar
Risk Assessment Branch 1, Health Effects Division (7509P) **Date:** 3/14/13
Template version 09/11

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study – Rat OCSPP 870.3800; OECD 416.

PC CODE: 417300 **DP BARCODE:** D409997

TXR#: 0056606 **CAS No:** 1071-83-6

TEST MATERIAL (PURITY): Glyphosate (95.7% a.i.)

SYNONYMS: *N*-(phosphonomethyl)glycine

CITATION: Dhinsa, N.K.; Watson, P; Brooks, P.N. (2007). Glyphosate technical: dietary two generation reproduction study in the rat. Safepharm Laboratories Limited, Derbyshire, UK. SPL Project Number: 2060/0013, November 6, 2007. MRID 48865101. Unpublished.

Dhinsa, N.K.; Watson, P; Brooks, P.N. (2007). Glyphosate technical: dietary two generation reproduction study in the rat; pages 301-667. Safepharm Laboratories Limited, Derbyshire, UK. SPL Project Number: 2060/0013, November 6, 2007. MRID 48865102. Unpublished.

Dhinsa, N.K.; Watson, P; Brooks, P.N. (2012). Glyphosate technical: dietary two generation reproduction study in the rat; amendment to final report. Safepharm Laboratories Limited, Derbyshire, UK. SPL Project Number: 2060/0013, June 18, 2012. MRID 48865103. Unpublished.

Dhinsa, N.K.; Watson, P; Brooks, P.N. (2012). Glyphosate technical: dietary two generation reproduction study in the rat; amendment to final report. Safepharm Laboratories Limited, Derbyshire, UK. SPL Project Number: 2060/0013, June 18, 2012. MRID 48865104. Unpublished.

Dhinsa, N.K.; Watson, P; Brooks, P.N. (2012). Glyphosate technical: dietary two generation reproduction study in the rat; cortical vacuolation historical data. Safepharm Laboratories Limited, Derbyshire, UK. SPL Project Number: 2060/0013, June 18, 2012. MRID 48865105. Unpublished.

SPONSOR: Nufarm Asia Sdn Bhd, Subang Jaya, Malaysia

TEST ORDER #: CON-417300-23

EXECUTIVE SUMMARY: In a 2-generation reproduction study (MRIDs 48865101 through

48865105) glyphosate (95.7% a.i. Lot # H05H016A) was administered to 28 Sprague Dawley [Crl:CD(SD) IGS BR] rats/sex/dose level in the diet at doses of 0, 1500, 5000 and 15,000 ppm (equivalent to 0/0, 121/126, 408/423, and 1234/1273 mg/kg/day in males/females during pre-mating) for two successive generations with one litter per generation. The P generation animals were fed the test diets for ten weeks prior to mating to produce the F1 litters. The F1 litters were not standardized; litter sizes ranged from 4 to 21 pups. On post-natal day (PND) 21, 24 pups/sex/dose level were selected and fed the same test diet as their parents for ten weeks prior to mating to produce the F2 litters.

No treatment-related effects were observed on mortality, clinical signs, body weights, body weight gains, food consumption, food efficiency, or gross or microscopic pathology in either the P or F1 generation.

One 1500 ppm P male was euthanized for humane reasons on Day 84 due to the presence of a fluid-filled mass (approximately 5 × 3 cm) around the throat. One 5000 ppm P female was killed *in extremis* on Day 103 following a suspected prolonged parturition (blood observed around the vagina and five dead fetuses *in utero*). One 15,000 ppm P female was found dead on Day 97 with a red, fluid-filled uterus containing nine underdeveloped dead fetuses and seven late death fetuses, and pallor of the liver, brain, and kidneys. This death was suspected to be due to complications during parturition. One control F1 female was euthanized for humane reasons on Day 99 following the observation of severe clinical signs (pallor of the extremities, lethargy, piloerection, hunched posture, and ano-genital staining) and was found to have two dead fetuses and ten placentae in the right uterine horn, dark kidneys, an enlarged fluid-filled pancreas, and mottled appearance of the liver at necropsy. All other animals survived to scheduled termination.

Absolute and relative (to body) liver weights were increased ($p<0.05$) by 8-13% in both the P and F1 females at 15,000 ppm. Additionally at this dose level, absolute and relative kidney weights were increased ($p<0.01$) by 7-11% in the P females.

The LOAEL for parental toxicity was not observed. The NOAEL is 15,000 ppm (equivalent to 1234/1273 mg/kg/day in males/females during pre-mating).

There were no effects of treatment on the mean numbers of corpora lutea and implantations, pre- and post-implantation loss, numbers of pups born, litter size on PND 1, 4, 7, 14, and 21, live birth, viability, and lactation indices, and sex ratio at birth and on PND 1 and 21, clinical signs of toxicity, litter weights, pup body weights, developmental landmarks or reflexes, age and body weight at attainment of VO, ano-genital distance, or brain, spleen or thymus weights in either the F1 or F2 offspring.

The 15,000 ppm F1 male pups had a delay ($p<0.01$) of 2.9 days in attaining complete PPS (PND 45.9 treated vs. PND 43.0 controls), along with a 10% increase ($p<0.01$) in body weight at attainment.

The LOAEL for offspring toxicity is 15,000 ppm (equivalent to 1234/1273 mg/kg/day in males/females during pre-mating), based on delayed age and increased weight at attainment of PPS. The NOAEL is 5000 ppm (equivalent to 408/423 in males/females during pre-mating).

The number of males impregnating their female partner, and the numbers of females not pregnant, with no litters observed, with total litter loss, and rearing their litters to weaning were similar in all groups. The precoital intervals were similar across all groups with the majority of pairs mating during the first four days of the mating period. Gestation duration, mean estrous cycle length and periodicity, follicle number, and sperm parameters were unaffected by treatment.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 15,000 ppm (equivalent to 1234/1273 mg/kg/day in males/females during pre-mating).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OCSPP 870.3800; OECD 416) for a two-generation reproduction study in the rat.

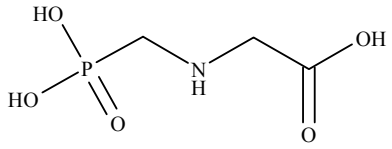
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided. A Flagging Statement was not provided, but should have been included to meet the requirements of 40 CFR 158.34.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material — Glyphosate

Description: Technical, white crystalline solid
Lot/batch #: H05H016A
Purity: 95.7% a.i.
Compound stability: Stable in the diet for up to six weeks at room temperature under dark conditions
CAS # of TGAI: 1071-83-6
Structure:



2. Vehicle and/or positive control: Diet

3. Test animals

Species: Rat
Strain: Sprague-Dawley Crl:CD(SD) IGS BR
Age at study initiation: Approximately 8 weeks
Wt. at study initiation: 138-257 g males
140-195 g females
Source: Charles River (UK) Limited, Margate, Kent, UK
Housing: Initially, all rats were housed in groups of up to four in suspended polypropylene cages with stainless steel grid floors and tops over polypropylene trays lined with absorbent paper. During mating, animals were transferred to similar cages on a one male:one female basis within each dose group. After mating, males were returned to their original cages. Mated females were housed individually during gestation and lactation in polypropylene cages with solid floors and stainless steel lids, and softwood flake bedding.
Diet: PMI 5002 Certified Rodent Diet (meal form; BCM IPS Ltd., London, England), *ad libitum*
Water: Tap water, *ad libitum*
Environmental conditions: **Temperature:** 21±2°C
Humidity: 55±15%
Air changes: ≥15/h
Photoperiod: 12h light/12 h dark
Acclimation period: At least 14 days

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: Not provided (approximately November, 2005 through August, 2006)

2. Mating procedure: Mating was accomplished by pairing one male with one female of the same dose group for up to 21 days. Cage tray-liners were checked each morning for the presence of ejected copulation plugs, and each female was examined for the presence of a copulation plug in the vagina. A vaginal smear was prepared for each female and the presence of sperm was recorded. The day on which positive evidence of mating (sperm detected in the vaginal smear and/or vaginal plug *in situ*) was recorded was designated as gestation day (GD) 0 (not stated, but assumed by the reviewers). The males were subsequently returned to their original holding cages; mated females were housed individually.

3. **Study schedule:** The P generation animals were fed the test diets for ten weeks prior to mating to produce the F1 litters. Females were allowed to litter normally and the day on which delivery was complete was designated as lactation day (LD) 0. Litters were not standardized. At weaning on PND 21, 24 pups/sex/dose group were randomly selected from the litters to become the F1 parents, and were fed the test diets for ten weeks prior to mating to produce the F2 litters.

4. **Animal assignment:** The P animals were randomly assigned (stratified by body weight) to the test groups presented in Table 1.

TABLE 1. Animal assignment [a]

Test group	Dose (ppm) [b]	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	28	28	24	24
Low (LDT)	1500	28	28	24	24
Mid (MDT)	5000	28	28	24	24
High (HDT)	15,000	28	28	24	24

a Data were obtained from page 19 of the study report.
b Exposure to the test substance was continuous throughout the study.

5. **Dose selection rationale:** It was stated that based on a previously conducted subchronic oral toxicity study (Coles *et al*., 1996[1]; not provided), dietary concentrations of 1500, 5000 and 15,000 ppm were selected. The limit dose (1000 mg/kg/day) was expected to be achieved at the 15,000 ppm dose level. No additional information was provided.

6. **Dosage preparation and analysis:** Formulations were prepared approximately weekly by mixing an appropriate amount of glyphosate with a small amount of basal diet to form a premix. The premix was then added to a larger amount of basal diet and mixed to yield the desired final concentration. Homogeneity (three areas) and stability analyses were performed on all concentrations of the first dietary mixtures. Stability of the test substance in the basal diet was evaluated for six weeks at room temperature. Concentration analyses were performed on all concentrations weekly for the first four weeks, and then approximately monthly for the remaining treatment period.

Results

Homogeneity (%CV): 0.84-3.32%

Stability (% of time 0): 99-103%

Concentration (% nominal): 83-102%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

1 Coles, L.J., Thomas, O.N., Bartlett, A.J., Brooks, P.N. (1996) Technical Glyphosate; Ninety Day Subchronic Oral (Dietary) Toxicity Study in the Rat, SPL 0434/016

C. OBSERVATIONS

1. Parental animals: All animals were examined for clinical signs of toxicity once daily. Individual body weights were recorded for P males on Day 1 (prior to treatment) and then weekly for P and F1 males until termination. P and F1 females were weighed daily until mating was confirmed. Body weights for females showing evidence of mating were recorded on GD 0, 7, 14 and 21. Females with live litters were weighed on LD 1, 4, 7, 14 and 2. Food consumption (g/rat/day) was recorded weekly for all cages, as well as for GD 0-7, 7-14 and 14-21, and LD 1-4, 4-7, 7-14, and 14-21 for the females. Estrus cyclicity and duration were determined by examination of daily vaginal smears over a three week period prior to mating, and estrus cycle stage was determined just prior to termination. Sperm was collected at necropsy from the left testis and epididymis for evaluation of motility, morphology, and sperm count. Clinical chemistry parameters were not examined.

2. Litter observations: According to the report, the following litter observations (X) were made (see Table 2).

TABLE 2. F_1/F_2 Litter Observations [a]

Observation	Time of observation (lactation day)				
	Day 1	Day 4 [b]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X
Pup weight	X	X	X	X	X
External alterations	X	X	X	X	X
Number of dead pups	X	X	X	X	X
Sex of each pup (M/F)	X	X			X

a Data obtained from pages 23 and 24 in the study report.
b Litters were not standardized

The litters were not standardized. All live offspring were observed for the detachment and unfolding of pinna, incisor eruption, and eyelid separation, and assessed for response to stimuli by assessing surface righting reflex on PND 1 and air righting reflex on PND 17. Pupillary reflex and auditory startle response were performed on PND 21. At each examination period (PND 1, 4, 7, 14 and 21) litters were examined for abnormal behavior and appearance. F1 males and females selected for mating were examined for preputial separation (PPS) or vaginal opening (VO) daily beginning on PND 21 until achievement. Age and body weight at attainment of criterion was recorded.

3. Postmortem observations

a. Parental animals: Surviving adult females were killed by an intravenous overdose of sodium pentobarbitone followed by exsanguination on PND 21. Following the termination of all adult females and offspring, all surviving males were killed by intravenous overdose of sodium pentobarbitone followed by exsanguination. All animals were subjected to a gross necropsy (external and internal examinations), and any macroscopic abnormalities were recorded.

The following tissues were weighed (X) from parental rats terminated as scheduled.

Additionally, the (XX) tissues were fixed in 10% buffered formalin, except the right epididymis and testis which were fixed in Bouin's fluid for 48 h and then transferred to 70% industrial methylated spirit.

XX	Adrenals	XX	Pituitary
X	Brain	XX	Seminal vesicles (with coagulating gland and fluid)
X	Cauda epididymis (left)	X	Spleen
XX	Epididymides	XX	Testes
X	Kidneys	X	Thymus
X	Liver	X	Thyroid
XX	Ovaries	XX	Uterus (with cervix, oviducts, and vagina)
XX	Prostate	XX	Gross lesions and masses

All tissues from the control and 15,000 ppm P and F1 animals, and any animals dying during the study, were routinely processed, stained with hematoxylin and eosin, and examined microscopically. Treatment-related changes were observed in the adrenals of the F1 animals; therefore, histological examinations were also performed on the adrenals from the 1500 and 5000 ppm groups. In addition, the corpora lutea of all ovaries from pregnant females were counted. Uterine implantation sites were counted using a 0.5% ammonium polysulfide solution for staining when necessary.

b. **Offspring:** Surviving offspring not selected to become the F1 parents were terminated by carbon dioxide asphyxiation on PND 21 and subjected to gross postmortem examinations. Pups that died during the study were also necropsied, and any gross abnormalities were noted.

D. DATA ANALYSIS

1. **Statistical analyses:** Absolute and relative (to body) organ weights, body weight gains, litter weights, and offspring body weights were analyzed by linear regression analysis, followed by a one way analysis of variance (ANOVA) incorporating Levene's test for homogeneity of variance. Where variances were shown to be homogeneous, pairwise comparisons were conducted using Dunnett's test. When Levene's test was significant, the data were analyzed using the non-parametric Kruskal-Wallis ANOVA followed by a Mann-Whitney U test. Implantation loss, offspring sex ratio, and developmental landmarks and reflex responses were also analyzed by these non-parametric tests. Histopathology data were analyzed using a Chi-squared analysis for differences in the incidence of lesions occurring with an overall frequency of 1 or greater, or a Kruskal-Wallis one-way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions. Statistical significance was denoted at $p<0.05$, $p<0.01$, and $p<0.001$. The statistical methods were considered appropriate.

2. **Indices**

Reproductive indices: The following reproductive indices were calculated by the performing laboratory from breeding and parturition records of animals in the study.

$$\text{Mating Index (\%)} = \frac{\text{Number of animals mated}}{\text{Number of animals paired}} \times 100$$

$$\text{Pregnancy Index (\%)} = \frac{\text{Number of pregnant females}}{\text{Number of animals mated}} \times 100$$

$$\text{Parturition Index (\%)} = \frac{\text{Number of females delivering live offspring}}{\text{Number of pregnant females}} \times 100$$

Offspring indices: The following indices were calculated by the performing laboratory from lactation records of litters in the study.

$$\text{Live Birth Index (\%)} = \frac{\text{Number of offspring alive on Day 1}}{\text{Number of offspring born}} \times 100$$

$$\text{Viability Index 1 (\%)} = \frac{\text{Number of offspring alive on Day 4}}{\text{Number of offspring alive on Day 1}} \times 100$$

$$\text{Viability Index 2 (\%)} = \frac{\text{Number of offspring alive on Day 7}}{\text{Number of offspring alive on Day 4}} \times 100$$

$$\text{Viability Index 3 (\%)} = \frac{\text{Number of offspring alive on Day 14}}{\text{Number of offspring alive on Day 7}} \times 100$$

$$\text{Viability Index 4 (\%)} = \frac{\text{Number of offspring alive on Day 21}}{\text{Number of offspring alive on Day 14}} \times 100$$

$$\text{Viability Index 5 (\%)} = \frac{\text{Number of offspring alive on Day 21}}{\text{Number of offspring alive on Day 1}} \times 100$$

$$\text{Sex Ratio (\% males)} = \frac{\text{Number of male offspring}}{\text{Total number of offspring}} \times 100$$

Implantation losses: Pre-implantation loss and post-implantation loss were calculated as follows.

$$\text{Pre} - \text{implantation loss (\%)} = \frac{\text{Number of corpora lutea} - \text{Number of implantation sites}}{\text{Number of corpora lutea}} \times 100$$

$$\text{Post} - \text{implantation loss} = \frac{\text{Number of implantation sites} - \text{Number of offspring}}{\text{Number of implantation sites}} \times 100$$

3. **Historical control data:** Historical control data were not provided in MRID 48865101; however, MRID 48865105 provided historical control data on the incidence of adrenal cortical vacuolation in Sprague-Dawley rats from nine one- or two-generation reproductive studies performed between 2004 and 2007 at Harlan Laboratories.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

a. **Mortality:** There were no treatment-related mortalities in either the P or F1 generation. One 1500 ppm P male was euthanized for humane reasons on Day 84 due to the presence of a fluid-filled mass (approximately 5 × 3 cm) around the throat. One 5000 ppm P female was killed *in extremis* on Day 103 following a suspected prolonged parturition (blood observed around the vagina and five dead fetuses *in utero*). One 15,000 ppm P female was found dead on Day 97 with a red, fluid-filled uterus containing nine underdeveloped dead fetuses and seven late death fetuses, and pallor of the liver, brain, and kidneys. This death was suspected to be due to complications during parturition. One control F1 female was euthanized for humane reasons on Day 99 following the observation of severe clinical signs (pallor of the extremities, lethargy, piloerection, hunched posture, and ano-genital staining) and was found to have two dead fetuses and ten placentae in the right uterine horn, dark kidneys, an enlarged fluid-filled pancreas, and mottled appearance of the liver at necropsy. All other animals survived to scheduled termination.

b. **Clinical signs:** There were no treatment-related clinical signs of toxicity. Commonly noted findings included incidents of red/brown staining of the body, generalized fur loss, scab formation, and red/brown staining on the cage tray-liners in the control and treatment groups. All other clinical signs occurred sporadically and/or in small numbers (1-4 rats) of rats in both generations throughout the study.

2. Body weight, body weight gain, food consumption, and food efficiency

a. **Pre-mating:** Pre-mating body weights, body weight gains, and food consumption are presented in Tables 3a and b. In the P generation, there were no effects of treatment observed on body weights, body weight gains, food consumption, or food efficiency in the males throughout treatment, or in the females during pre-mating (Table 3a).

TABLE 3a. Selected mean (±SD) body weights, body weight gains, and food consumption in the P generation during pre-mating[a]

Observation/study day		Dose Group (ppm)			
		0	1500	5000	15,000
P Males (n=27-28)					
Body weight (g)	Week 1	189±19	193±22	194±18	194±22
	Week 10	490±51	505±46	501±36	483±55
	Week 19	585±59	607±58	606±49	584±72
Body weight gain (g)	Weeks 1-10	301±49	312±41	307±38	289±47
	Weeks 1-19	396±58	414±51	412±55	390±64
Food consumption (g/rat/day)	Week 1	24±1	24±1	25±1	25±1
	Week 10	28±1	29±1	30±2	28±2
	Week 18	27±1	29±2	29±1	28±1
P Females (n=28)					
Body weight (g)	Week 1	168±14	167±14	169±14	168±14
	Week 11	299±22	299±31	309±31	289±26
Body weight gain (g)	Weeks 1-11	131±15	132±23	141±23	121±16
Food consumption (g/rat/day)	Week 1	17±1	18±1	18±1	17±1
	Week 10	19±1	19±1	20±1	19±1

a Data were obtained from Tables 6, 7, 10, and 11 on pages 66, 68, 72-73, 84, and 86 of the study report.

In the F1 generation, there were no effects of treatment observed on body weights, body

weight gains, food consumption, or food efficiency in the males throughout treatment, or in the females during pre-mating (Table 3b). In the 15,000 ppm males, weekly body weight gains were decreased ($p<0.05$) by 35% during Week 13, but increased ($p<0.01$) by 350% during Week 18. Cumulative (Week 1-19) body weight gains for this group were similar to controls. In the females, weekly body weight gains were decreased ($p<0.05$) by 50% at 1500 ppm and by 63% at 15,000 ppm during Week 10; however, cumulative (Week 1-10) body weight gains were similar to controls in both groups. These transient alterations in weekly body weight gains were not considered adverse.

TABLE 3b. Selected mean (±SD) body weights, body weight gains, and food consumption in the F1 generation during pre-mating[a]

Observation/study day		Dose Group (ppm)			
		0	1500	5000	15,000
F1 Males (n=23-24)					
Body weight (g)	Week 1	84±10	84±9	88±12	82±14
	Week 10	483±37	484±48	490±42	466±31
	Week 19	592±49	589±63	603±58	572±43
Body weight gain (g)	Weeks 1-10	399±31	400±44	402±35	383±31
	Weeks 1-19	507±44	506±60	515±53	490±46
Food consumption (g/rat/day)	Week 1	16±1	16±1	16±1	15±1
	Week 10	29±1	29±2	30±2	29±2
	Week 18	29±1	29±2	30±1	29±2
F1 Females (n=23-24)					
Body weight (g)	Week 1	83±9	83±8	89±12	85±11
	Week 11	285±28	284±24	303±30	280±22
Body weight gain (g)	Weeks 1-11	202±26	202±25	214±25	196±23
Food consumption (g/rat/day)	Week 1	14±1	14±0	15±1	14±1
	Week 10	20±1	20±1	21±1	20±1

a Data were obtained from Tables 6, 7, 10, and 11 on pages 69, 71, 74-75, 85, and 87 of the study report.

There were no effects of treatment observed on body weights, body weight gains, or food consumption in the P or F1 females during gestation (Table 3c).

TABLE 3c. Mean (±SD) body weights and body weight gains during gestation[a]

Observation/study day		Dose Group (ppm)			
		0	1500	5000	15,000
P Females (n=23-28)					
Body weight (g)	GD 0	302±21	307±31	312±31	295±34
	GD 7	332±24	337±31	344±29	326±31
	GD 14	363±25	369±32	377±30	356±32
	GD 21	459±36	463±40	486±39	457±39
Body weight gain (g)	GD 1-21	157±22	156±25	174±26	162±30
F1 Females (n=22-24)					
Body weight (g)	GD 0	290±27	291±24	304±27	281±27
	GD 7	325±26	328±30	338±25	317±27
	GD 14	358±27	357±25	371±25	348±28
	GD 21	456±41	458±32	475±31	453±31
Body weight gain (g)	GD 1-21	168±22	167±17	171±20	171±15

a Data were obtained from Table 8 on pages 76-79 of the study report.

There were no effects of treatment observed on body weights, body weight gains, or food consumption in the P or F1 females during lactation (Table 3d). During the last week of

lactation, both the P and F1 15,000 ppm females had smaller ($p<0.01$) body weight losses (P females: −8g treated vs. −23g controls; F1 females: −4g treated vs. −16g controls). However, these reduced weight losses were not considered adverse.

TABLE 3d. Mean (±SE) body weights and body weight gains during lactation[a]

Observation/study day		Dose Group (ppm) 0	1500	5000	15,000
P Females (n=26-27)					
Body weight (g)	LD 1	336±28	343±33	351±30	333±31
	LD 4	351±28	360±31	368±27	351±31
	LD 7	373±26	375±30	387±28	369±32
	LD 14	373±22	378±31	387±26	370±29
	LD 21	349±20	352±27	364±26	362±28
Body weight gain (g)	LD 1-21	13±17	9±16	12±15	29±15
F1 Females (n=22-24)					
Body weight (g)	LD 1	342±31	343±27	355±30	332±26
	LD 4	356±31	357±27	372±28	349±25
	LD 7	365±31	373±26	382±24	359±35
	LD 14	374±29	377±27	387±22	370±25
	LD 21	358±24	356±27	370±26	365±24
Body weight gain (g)	LD 1-21	16±16	12±20	16±17	33±13

a Data were obtained from Table 9, pages 80-83 of the study report.

3. Test substance intake: Test substance intakes (mg/kg/day) during pre-mating are presented in Table 4.

TABLE 4. Mean test compound intake (mg/kg/day) during pre-mating[a]

Group		Dose Group (ppm) 0	1500	5000	15,000
P generation males	Weeks 1-10[b]	0	110	373	1116
F1 generation males	Weeks 1-10[b]	0	133	444	1353
Males average mean intake[c]		**0**	**121**	**408**	**1234**
P generation females	Weeks 1-10[b]	0	116	389	1165
F1 generation females	Weeks 1-10[b]	0	136	457	1380
Females average mean intake[b]		**0**	**126**	**423**	**1273**

a Data were obtained from page 34 and Table 2 on pages 51-54 of the study report.
b Calculated by the reviewers.
c Calculated by reviewers from data presented in this table.

During gestation, mean achieved concentrations of pregnant females were 108, 359, and 1109 mg/kg/day at 1500, 5000, and 15,000 ppm, respectively. During lactation, mean achieved concentrations of females were 356, 1142, and 3574 mg/kg/day at 1500, 5000 and 15,000 ppm, respectively.

4. Reproductive function

a. Estrous cycle length and periodicity: Mean estrous cycle length was not calculated. There were no effects of treatment on the type or number of females with irregular estrous cycles in either generation. There was a slight increase in the number of F1 females having

irregular estrous cycles at 5000 ppm and above (5-6 treated vs. 2 controls); however, it was stated that these higher values were not reflected in the pre-coital intervals, suggesting there was no difference in mating performance. These differences were considered to be incidental in nature.

b. **Follicle number (F1):** There were no treatment-related effects on follicle number. The number of large follicles was increased ($p<0.01$) in the 15,000 ppm F1 females (11.0 treated vs. 8.0 controls); however, as there were no differences in the number of offspring produced, this finding was considered equivocal.

c. **Sperm measures:** There were no treatment-related effects on the concentration, motility, or morphology of sperm in either generation. There were no abnormal sperm observed in any group in either generation. The number of homogenization-resistant spermatid present in the cauda epididymis of the 15,000 ppm P males was lower ($p<0.05$; ↓23%) than the controls. However, the number of homogenization-resistant spermatid present in the testis of these animals was similar to controls. Therefore, the observed decrease was considered incidental.

5. **Reproductive performance:** Reproductive performance (Table 5) was not affected by treatment in either generation. The number of males impregnating their female partner, and the numbers of females not pregnant, with no litters observed, with total litter loss, and rearing their litters to weaning were similar in all groups. The precoital intervals were similar across all groups with the majority of pairs mating during the first four days of the mating period. Gestation durations were similar across all groups.

TABLE 5. Reproductive performance[a]

Observation	Dose Group (ppm)			
	0	1500	5000	15,000
P generation				
Males paired	28	28	28	28
Males impregnating female partner	27	27	28	28
Females paired	28	28	28	28
Females not pregnant	1	1	0	0
Females with no litter observed	1	0	0	0
Females with total litter loss	0	0	0	1
Females rearing young to weaning	26	27	28	26
Pre-coital interval (mean±SD days)[b]	3.04±3.05	2.93±0.96	3.32±3.56	3.82±4.18
Gestation length (mean±SD days)[b]	22.40±0.47	22.46±0.44	22.59±0.45	22.57±0.49
F1 generation				
Males paired	24	24	24	24
Males impregnating female partner	23	23	24	23
Females paired	24	24	24	24
Females not pregnant	1	1	0	1
Females with no litter observed	0	0	0	0
Females with total litter loss	0	0	0	0
Females rearing young to weaning	22	23	24	23
Pre-coital interval (mean±SD days)[b]	2.63±1.06	3.13±2.86	3.35±1.61	2.96±2.42
Gestation length (mean±SD days)[b]	22.43±0.50	22.41±0.44	22.48±0.41	22.37±0.50

a Data were obtained from Table 1 on page 50 and Table 16 on pages 95-96 of the study report.
b Calculated by the reviewers.

6. Parental postmortem results

a. Organ weights: Selected organ weight data are presented in Table 6. Absolute and relative (to body) liver weights were increased ($p<0.05$) by 8-13% in both the P and F1 females at 15,000 ppm. Additionally at this dose level, absolute and relative kidney weights were increased ($p<0.01$) by 7-11% in the P females; kidney weights were not affected in the F1 females. Absolute kidney weight was increased ($p<0.05$) in the 5000 ppm P females; however, relative kidney weight was similar to control at this dose level. Therefore, this increase was considered incidental. Absolute thyroid weight was increased ($p<0.05$) in the 5000 ppm P females; however, this increase was not considered treatment-related due to the lack of dose dependency.

In the 15,000 ppm F1 males, absolute brain weights were decreased ($p<0.01$) by 5% and absolute spleen weights were decreased ($p<0.05$) by 13%; however, the relative weights of these organs were similar to controls. Therefore, these decreases were considered incidental. In the 1500 and 5000 ppm P males, relative thyroid weights were decreased ($p<0.05$) by 22-24%; however, these decreases were not considered treatment-related due to the lack of dose dependency. There were no other changes observed in organ weights.

TABLE 6. Mean (±SD) liver and kidney weights in female rats treated with glyphosate [a]

Observation/study day	Dose Group (ppm)			
	0	1500	5000	15,000
P generation (n=26-27)				
Terminal body weight (g)	349±20	352±27	364±26	362±28
Absolute liver weight (g)	15.0328±1.0493	15.1465±1.4948	15.8791±1.7649	16.9704±1.7620**(↑13)
Relative (to body) liver weight (%)	4.3103±0.2864	4.3027±0.3435	4.3570±0.2810	4.6806±0.2977***(↑9)
Absolute kidney weight (g)	2.4315±0.1706	2.5395±0.1602	2.5654±0.2361*(↑6)	2.7096±0.2203***(↑11)
Relative (to body) kidney weight (%)	0.6977±0.0548	0.7233±0.0560	0.7062±0.0592	0.7490±0.0521** (↑7)
F1 Generation (n=22-24)				
Terminal body weight (g)	358±24	356±27	370±26	365±24
Absolute liver weight (g)	16.4887±2.0275	16.3848±1.7744	17.2591±2.0969	18.0724±1.2434* (↑10)
Relative (to body) liver weight (%)	4.5970±0.4038	4.6047±0.2858	4.6543±0.3628	4.9591±0.3130** (↑8)
Absolute kidney weight (g)	2.6792±0.4137	2.5777±0.2776	2.8124±0.5326	2.7660±0.2616
Relative (to body) kidney weight (%)	0.7483±0.1070	0.7257±0.0647	0.7585±0.1229	0.7578±0.0517

a Data were obtained from Tables 31 and 32 on pages 141-144 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Significantly different from controls; $p<0.05$
** Significantly different from controls; $p<0.01$
*** Significantly different from controls; $p<0.001$

b. Pathology

1. Macroscopic examination: There were no treatment-related findings observed at necropsy. All macroscopic findings were low in incidence and occurred sporadically in all dose groups.

2. Microscopic examination: Microscopic findings are presented in Table 7. In the P males, minimal to slight cortical vacuolation of the adrenal glands was observed at 15,000 ppm (12 treated vs. 8 controls). In the F1 males, the number of rats with cortical vacuolation was decreased ($p<0.05$) at 5000 and 15,000 ppm compared to controls (8-10 treated vs. 17 controls); the severity of the finding was slightly increased at 15,000 ppm compared to

controls (minimal to moderate treated vs. minimal to slight controls). However, these findings fell within the range of historical control data (MRID 48865105). All other microscopic findings were low in incidence and severity or commonly observed in rats of this strain and age.

TABLE 7. Incidence of cortical vacuolation in the adrenal glands of male rats treated with glyphosate [a]

Histopathological finding		Dose Group (ppm)			
		0	1500	5000	15,000
P generation (n=27-28)					
Adrenal gland					
Cortical vacuolation	Total	8	ND	ND	12
	Minimal	6			8
	Slight	2			4
F1 Generation (n=24)					
Adrenal gland					
Cortical vacuolation	Total	17	10	8*	10*
	Minimal	10	6	6	7
	Slight	7	4	2	2
	Moderate	0	0	0	1

a Data were obtained from Table 39 on pages 154 and 156 of the study report.
ND No data
* Significantly different from controls; $p<0.05$

B. OFFSPRING

1. Viability and clinical signs: Litter parameters are presented in Table 8. There were no effects of treatment on either the F1 or F2 offspring from birth throughout lactation. The mean numbers of corpora lutea and implantations, pre- and post-implantation loss, numbers of pups born, litter size on PND 1, 4, 7, 14, and 21, live birth, viability, and lactation indices, and sex ratio at birth and on PND 1 and 21 were similar across all groups.

No clinical signs of toxicity were observed in either the F1 or F2 pups.

TABLE 8. Litter parameters for F_1 and F_2 generations [a]

Observation		Dose group (ppm)			
		0	1500	5000	15,000
F_1 Generation					
Mean (±SD) number of corpora lutea		17.4±1.9	15.9±2.3	17.6±2.1	17.2±2.1
Mean (±SD) implantation sites		16.3±2.0	14.8±3.1	16.3±1.9	15.9±2.2
Pre-implantation loss (%)		6.5±6.6	8.0±12.0	6.7±5.5	7.4±7.0
Post-implantation loss (%)		5.1±6.5	4.9±6.3	4.2±4.9	5.1±8.3
Mean (±SD) number born		15.4±1.7	14.1±3.1	15.7±2.2	15.1±2.2
Mean (±SD) litter size	PND 1	15.2±1.6	14.0±3.1	15.4±2.1	14.7±2.2
	PND 4[b]	15.2±1.6	13.8±3.1	15.4±2.1	14.5±2.1
	PND 7	15.1±1.7	13.8±3.1	15.2±2.0	14.3±2.0
	PND 14	14.8±1.7	13.5±2.9	13.9±2.4	13.8±2.9
	PND 21	14.8±1.8	13.4±2.9	13.8±2.5	13.8±2.9
Mean (±SD) live birth index (%)[c]		98.8±2.9	99.2±3.0	98.2±3.3	97.2±4.3
Mean (±SD) viability index (%)[d]		99.5±2.5	98.9±2.9	100±0.0	98.7±2.7
Mean (±SD) lactation index(%)[e]		97.2±5.0	96.6±5.4	90.5±14.8	94.9±16.1
Mean (±SD) sex ratio (% male)	Birth	47.1±13.4	44.1±11.1	50.6±11.7	50.6±12.7
	PND 1	47.0±13.5	43.9±11.3	50.4±11.3	50.7±13.6
	PND 21	46.7±14.2	44.0±11.3	49.7±13.2	51.0±13.1
F_2 Generation					
Mean (±SD) number of corpora lutea		18.0±1.9	17.0±1.7	18.2±2.5	17.5±1.7
Mean (±SD) implantation sites		15.5±2.6	15.4±2.1	15.7±2.4	16.0±1.7
Pre-implantation loss (%)		13.5±14.4	9.3±8.0	13.1±12.1	8.6±5.7
Post-implantation loss (%)		12.3±15.6	5.4±7.1	6.0±8.7	7.0±6.4
Mean (±SD) number born		13.8±3.5	14.7±2.3	14.9±2.8	15.1±1.8
Mean (±SD) litter size	PND 1	13.7±3.4	14.5±2.3	14.5±3.2	14.9±1.8
	PND 4[b]	13.7±3.4	14.3±2.2	14.5±3.1	14.7±1.9
	PND 7	13.5±3.4	14.3±2.2	14.2±2.9	14.6±2.0
	PND 14	12.4±3.8	13.9±2.1	12.9±3.8	13.6±2.7
	PND 21	12.2±4.0	13.8±2.0	12.8±3.9	13.6±2.7
Mean (±SD) live birth index (%)[c]		99.1±2.3	98.3±3.5	97.1±8.7	98.6±4.1
Mean (±SD) viability index (%)[d]		100.0±0.0	99.2±2.1	99.6±1.9	99.1±2.5
Mean (±SD) lactation index(%)[e]		89.5±19.1	96.0±8.2	89.0±21.1	92.1±17.0
Mean (±SD) sex ratio (% male)	Birth	48.2±17.2	47.9±12.0	55.6±14.7	48.9±14.2
	PND 1	48.3±17.1	47.8±12.3	55.4±14.7	49.2±13.9
	PND 21	48.3±18.2	47.3±11.8	53.7±16.1	48.3±14.7

a Data obtained from Tables 17-19 on pages 97-102 of the study report.
b Litters were not standardized
c Live birth index (%) = (# pups alive on PND 1/# pups born) × 100
d Viability index (%) = (# pups on PND 4 per litter/# live pups born per litter) × 100
e Lactation index (%) = (total # pups on PND 21 per litter/# live pups on PND 1 per litter) × 100

2. <u>Body weight</u>: Mean pup body weight data are presented in Table 9. There were no effects of treatment on litter weights or pup body weights in either the F1 or F2 generation.

TABLE 9. Mean (±SD) litter and pup weights (g) [a]

	Dose group (ppm)							
	0	1500	5000	15,000	0	1500	5000	15,000
PND	F1 Litters				F2 Litters			
1	107.3±8.2	99.2±20.9	109.4±15.2	103.4±12.3	99.1±22.4	102.8±14.9	106.0±18.0	105.0±11.7
4	156.1±10.9	145.4±29.9	156.5±20.0	148.2±15.3	144.5±31.2	154.2±20.3	154.1±23.1	152.9±17.6
7	225.2±16.7	213.2±40.7	222.2±25.6	213.6±23.4	206.7±44.4	224.8±28.6	221.3±31.9	218.8±21.8
14	407.5±26.0	388.1±70.9	395.8±49.7	380.7±70.4	361.0±94.9	406.4±43.1	379.2±90.3	378.9±61.0
21	660.8±46.4	634.8±115.9	634.3±88.4	606.7±115.1	576.8±154.0	661.6±71.7	605.9±149.5	603.3±97.3
	F1 Pups – male				F2 Pups – male			
1	7.3±0.5	7.4±0.6	7.4±0.7	7.3±0.5	7.7±0.8	7.3±0.4	7.6±0.9	7.3±0.7
4	10.6±0.9	10.9±1.0	10.5±1.3	10.6±1.2	11.3±1.6	11.1±1.0	11.2±1.8	10.7±1.3
7	15.3±1.5	16.0±1.7	15.1±2.0	15.4±1.9	16.3±2.9	16.2±1.8	16.4±3.0	15.5±2.0
14	28.3±2.9	29.8±3.3	29.2±4.3	28.4±3.3	31.4±4.8	30.2±3.7	31.0±4.8	29.1±3.6
21	46.1±4.8	49.0±5.5	47.5±6.5	45.3±5.6	50.9±8.2	49.8±5.4	50.2±8.6	46.5±6.9
	F1 Pups – female				F2 Pups – female			
1	6.9±0.5	7.0±0.6	6.9±0.7	6.9±0.6	7.2±0.8	6.9±0.4	7.2±0.8	6.9±0.6
4	10.2±0.9	10.5±1.0	10.0±1.4	10.2±1.3	10.5±1.6	10.5±0.8	10.7±1.7	10.2±1.3
7	14.8±1.5	15.5±1.8	14.4±2.4	14.8±1.8	15.4±2.9	15.6±1.6	15.6±2.6	14.8±1.8
14	27.2±2.9	28.9±3.6	28.3±3.9	27.5±3.2	28.7±4.3	29.1±3.5	29.3±4.7	27.5±3.1
21	44.1±4.6	47.2±5.9	45.5±6.4	43.9±5.8	47.9±7.5	47.1±5.3	47.6±7.9	44.1±6.2

a Data obtained from Table 17 on pages 97-98 and Table 20 on pages 103-106 of the study report.

3. Developmental landmarks and reflexes: There were no effects of treatment on developmental landmarks (pinna unfolding, incisor eruption, and eye opening) or reflexes (surface righting, mid-air righting, pupil reflex, and startle reflex) in either the F1 or F2 generation. A slightly lower ($p<0.05$) percentage of the treated F2 pups successfully demonstrated the mid-air righting reflex (94.2-98.6% treated vs. 100% controls); however, this finding was considered incidental due to the small magnitude of difference and lack of dose-dependency observed.

4. Sexual maturation (F_1): The 15,000 ppm F1 male pups had a delay ($p<0.01$) of 2.9 days in attaining complete PPS (PND 45.9 treated vs. PND 43.0 controls), along with a 10% increase ($p<0.01$) in body weight at attainment. There were no effects of treatment on age or weight at attainment of VO.

5. Ano-genital distance (F2): There were no effects of treatment observed on ano-genital distance on PND 1 in the F2 pups.

6. Offspring postmortem results

a. Organ weights: There were no effects of treatment observed on brain, spleen, or thymus weights in the F1 pups. Absolute thymus weights were decreased ($p<0.05$) by 20% in the 15,000 ppm F2 pups; however, relative thymus weights were similar to controls. Therefore, this finding was considered to be of no toxicological significance.

b. Pathology

1) Macroscopic examination: There were no treatment-related effects observed at necropsy of the unselected F1 pups or the F2 pups.

2) **Microscopic examination:** No microscopic examinations were performed on the unselected F1 pups or the F2 pups.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The oral administration of glyphosate to rats by dietary admixture at a maximum dose of 15,000 ppm for two successive generations resulted in possible treatment-related changes at 15,000 ppm. The effects were not considered to represent an adverse health effect; therefore, the NOAEL was considered to be 15,000 ppm for adult toxicity for the P and F1 generations. The NOAEL for reproductive and developmental toxicity was considered to be 15,000 ppm.

B. **REVIEWER COMMENTS**

1. **PARENTAL ANIMALS:** No treatment-related effects were observed on mortality, clinical signs body weights, body weight gains, food consumption, food efficiency, or gross or microscopic pathology in either the P or F1 generation.

One 1500 ppm P male was euthanized for humane reasons on Day 84 due to the presence of a fluid-filled mass (approximately 5 × 3 cm) around the throat. One 5000 ppm P female was killed *in extremis* on Day 103 following a suspected prolonged parturition (blood observed around the vagina and five dead fetuses *in utero*). One 15,000 ppm P female was found dead on Day 97 with a red, fluid-filled uterus containing nine underdeveloped dead fetuses and seven late death fetuses, and pallor of the liver, brain, and kidneys. This death was suspected to be due to complications during parturition. One control F1 female was euthanized for humane reasons on Day 99 following the observation of severe clinical signs (pallor of the extremities, lethargy, piloerection, hunched posture, and ano-genital staining) and was found to have two dead fetuses and ten placentae in the right uterine horn, dark kidneys, an enlarged fluid-filled pancreas, and mottled appearance of the liver at necropsy. All other animals survived to scheduled termination.

Absolute and relative (to body) liver weights were increased ($p<0.05$) by 8-13% in both the P and F1 females at 15,000 ppm. Additionally at this dose level, absolute and relative kidney weights were increased ($p<0.01$) by 7-11% in the P females.

The LOAEL for parental toxicity was not observed. The NOAEL is 15,000 ppm (equivalent to 1234/1273 mg/kg/day in males/females during pre-mating).

2. **OFFSPRING:** There were no effects of treatment on the mean numbers of corpora lutea and implantations, pre- and post-implantation loss, numbers of pups born, litter size on PND 1, 4, 7, 14, and 21, live birth, viability, and lactation indices, and sex ratio at birth and on PND 1 and 21, clinical signs of toxicity, litter weights, pup body weights, developmental landmarks or reflexes, age and body weight at attainment of VO, ano-genital distance, or brain, spleen or thymus weights in either the F1 or F2 offspring.

The 15,000 ppm F1 male pups had a delay ($p<0.01$) of 2.9 days in attaining complete PPS (PND 45.9 treated vs. PND 43.0 controls), along with a 10% increase ($p<0.01$) in body

weight at attainment.

The LOAEL for offspring toxicity is 15,000 ppm (equivalent to 1234/1273 mg/kg/day in males/females during pre-mating), based on delayed age and increased weight at attainment of PPS. The NOAEL is 5000 ppm (equivalent to 408/423 in males/females during pre-mating).

C. REPRODUCTIVE TOXICITY: The number of males impregnating their female partner, and the numbers of females not pregnant, with no litters observed, with total litter loss, and rearing their litters to weaning were similar in all groups. The precoital intervals were similar across all groups with the majority of pairs mating during the first four days of the mating period. Gestation duration, mean estrous cycle length and periodicity, follicle number, and sperm parameters were unaffected by treatment.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 15,000 ppm (equivalent to 1234/1273 mg/kg/day in males/females during pre-mating).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OCSPP 870.3800; OECD 416) for a two-generation reproduction study in the rat.

D. STUDY DEFICIENCIES:

- **In life study dates are not provided.**
- **The number of animals was reduced from 28 in the P generation to 24 in the F1 generation and there was no explanation for this change.**
- **The mean estrous cycle dates were not calculated.**